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# A single session of hyperbaric oxygen therapy demonstrates acute and long-lasting neuroplasticity effects in humans: a replicated, randomized controlled clinical trial

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Anna M Wahl <sup>1</sup>  
Daniel Bidstrup <sup>1</sup>  
Isabel G Smidt-Nielsen <sup>1</sup>  
Mads U Werner <sup>2</sup>  
Ole Hyldegaard <sup>1,3</sup>  
Per Rotbøll-Nielsen <sup>2</sup>

<sup>1</sup>Hyperbaric Unit, Department of Anesthesia, Head and Orthopedic Center, Rigshospitalet, Copenhagen University Hospitals, Copenhagen, Denmark; <sup>2</sup>Multidisciplinary Pain Center, Neuroscience Center, Rigshospitalet, Copenhagen University Hospitals, Copenhagen, Denmark; <sup>3</sup>Institute of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark

**Purpose:** Animal studies have demonstrated anti-inflammatory, and anti-nociceptive properties of hyperbaric oxygen therapy (HBOT). However, physiological data are scarce in humans. In a recent experimental study, the authors used the burn injury (BI) model observing a decrease in secondary hyperalgesia areas (SHA) in the HBOT-group compared to a control-group. Surprisingly, a long-lasting neuroplasticity effect mitigating the BI-induced SHA-response was seen in the HBOT-preconditioned group. The objective of the present study, therefore, was to confirm our previous findings using an examiner-blinded, block-randomized, controlled, crossover study design.

**Patients and methods:** Nineteen healthy subjects attended two BI-sessions with an inter-session interval of  $\geq 28$  days. The BIs were induced on the lower legs by a contact thermode ( $12.5 \text{ cm}^2$ ,  $47^\circ\text{C}$ , 420 s). The subjects were block-randomized to receive HBOT (2.4 ATA, 100%  $\text{O}_2$ , 90 min) or ambient conditions ([AC]; 1 ATA, 21%  $\text{O}_2$ ), dividing cohorts equally into two sequence allocations: HBOT-AC or AC-HBOT. All sensory assessments performed during baseline, BI, and post-intervention phases were at homologous time points irrespective of sequence allocation. The primary outcome was SHA, comparing interventions and sequence allocations.

**Results:** Data are mean (95% CI). During HBOT-sessions a mitigating effect on SHA was demonstrated compared to AC-sessions, ie,  $18.8 (10.5\text{--}27.0) \text{ cm}^2$  vs  $32.0 (20.1\text{--}43.9) \text{ cm}^2$  ( $P=0.021$ ), respectively. In subjects allocated to the sequence AC-HBOT a significantly larger mean difference in SHA in the AC-session vs the HBOT-session was seen  $25.0 (5.4\text{--}44.7) \text{ cm}^2$  ( $P=0.019$ ). In subjects allocated to the reverse sequence, HBOT-AC, no difference in SHA between sessions was observed ( $P=0.55$ ), confirming a preconditioning, long-lasting ( $\geq 28$  days) effect of HBOT.

**Conclusion:** Our data demonstrate that a single HBOT-session compared to control is associated with both acute and long-lasting mitigating effects on BI-induced SHA, confirming central anti-inflammatory, neuroplasticity effects of hyperbaric oxygen therapy.

**Keywords:** burns, hyperbaric oxygenation, inflammation, pathophysiology, secondary hyperalgesia

Correspondence: Anna M Wahl  
Hyperbaric Unit, Department of  
Anesthesia, Head and Orthopedic Center,  
Rigshospitalet, Copenhagen University  
Hospitals, Blegdamsvej 9, 2100  
Copenhagen, Denmark  
Tel +45 2 324 8933  
Email Annamygd@gmail.com

## Plain language summary

Following hyperbaric oxygen therapy in animals, studies demonstrate anti-inflammatory and analgesic effects. However, human studies are surprisingly scarce in this field. The authors in a recent novel study showed that hyperbaric oxygen therapy in humans was associated with a long-lasting reduction of pain sensitivity in the skin surrounding an injured area. The

objective of the present study was to further examine and validate these findings using an improved methodological design. An experimental first degree burn injury was induced on the lower leg in 19 healthy male subjects followed by an intervention of either hyperbaric oxygen therapy (2.4 ATA, 100% oxygen, 90 min) or normal ambient conditions (1 ATA, 21% oxygen [corresponds to conditions at sea level]) serving as a control. Quantitative sensory skin assessments were made at standardized time intervals. The test subjects received each treatment at study sessions separated by  $\geq 28$  days. The hyperbaric session was compared to the ambient control session allowing the subjects to be their own control. Data from the present study confirms our previous findings showing a long-lasting ( $\geq 1$  month) reduction of pain sensitivity in the skin around the burn injury. These findings indicate that hyperbaric oxygen therapy could be an interesting venue for clinical pain research in persistent postsurgical pain and phantom limb pain.

## Introduction

Hyperbaric oxygen therapy (HBOT) is a recognized treatment form<sup>1,2</sup> and has been considered an adjunctive treatment for chronic pain conditions.<sup>3,4</sup> Several experimental studies, using different methods and treatment dosages, have shown anti-inflammatory effects of HBOT,<sup>5–7</sup> but there is a paucity of studies investigating these effects in humans. A previous study,<sup>8</sup> carried out by the present authors, was the first in humans to demonstrate an ameliorating effect of HBOT on the pathophysiological consequences following a previously validated inflammatory burn injury (BI) model.<sup>9–11</sup> We demonstrated an attenuation of secondary hyperalgesia areas (SHA) in normal skin surrounding the injury, likely reflecting a central anti-nociceptive effect. Interestingly, a preconditioning, protective effect of HBOT on the development of secondary hyperalgesia was also seen, more than one month after the primary injury. Both phenomena indicated an effect on central sensitization.<sup>12,13</sup>

The original study was a randomized, controlled, crossover study with an open therapeutic design.<sup>8</sup> The objective of the present study was to replicate our findings, adding an improved single-blinded design, ie, blinding for examiner bias. The primary outcome was an assessment of SHA, a measure of central sensitization. The secondary outcomes were measures of peripheral inflammation and sensitization, ie edema, erythema, mechanical pain thresholds, and, thermal detection and pain thresholds. In addition, exploratory analyses of the combined data of the previous and present studies were made.

## Methods

### Approvals

The study was approved by the Committee of Health Research Ethics of the Capital Region of Denmark (no. H-6-2014-089), the Danish Data Protection Agency (no. 30-1332) and the Danish Health Authority (no. 2014-003858-14). The study was registered before patient enrollment at EudraCT (no. 2014-003858-14, <https://www.clinicaltrialsregister.eu/ctr-search/search?query=2014-003858-14>), principal investigator: Ole Hyldegaard, registration date: September 23rd 2014. The study was also registered in clinicaltrials.gov (clinicaltrials.gov identifier: NCT02397343, <https://clinicaltrials.gov/ct2/show/NCT02397343?term=NCT02397343&rank=1>). The study complied with regulations of Good Clinical Practice (GCP) and was monitored by the GCP-unit of Copenhagen University Hospitals. The manuscript adheres to the applicable CONSORT guidelines (Figure 1). No amendment to the original protocol was submitted.

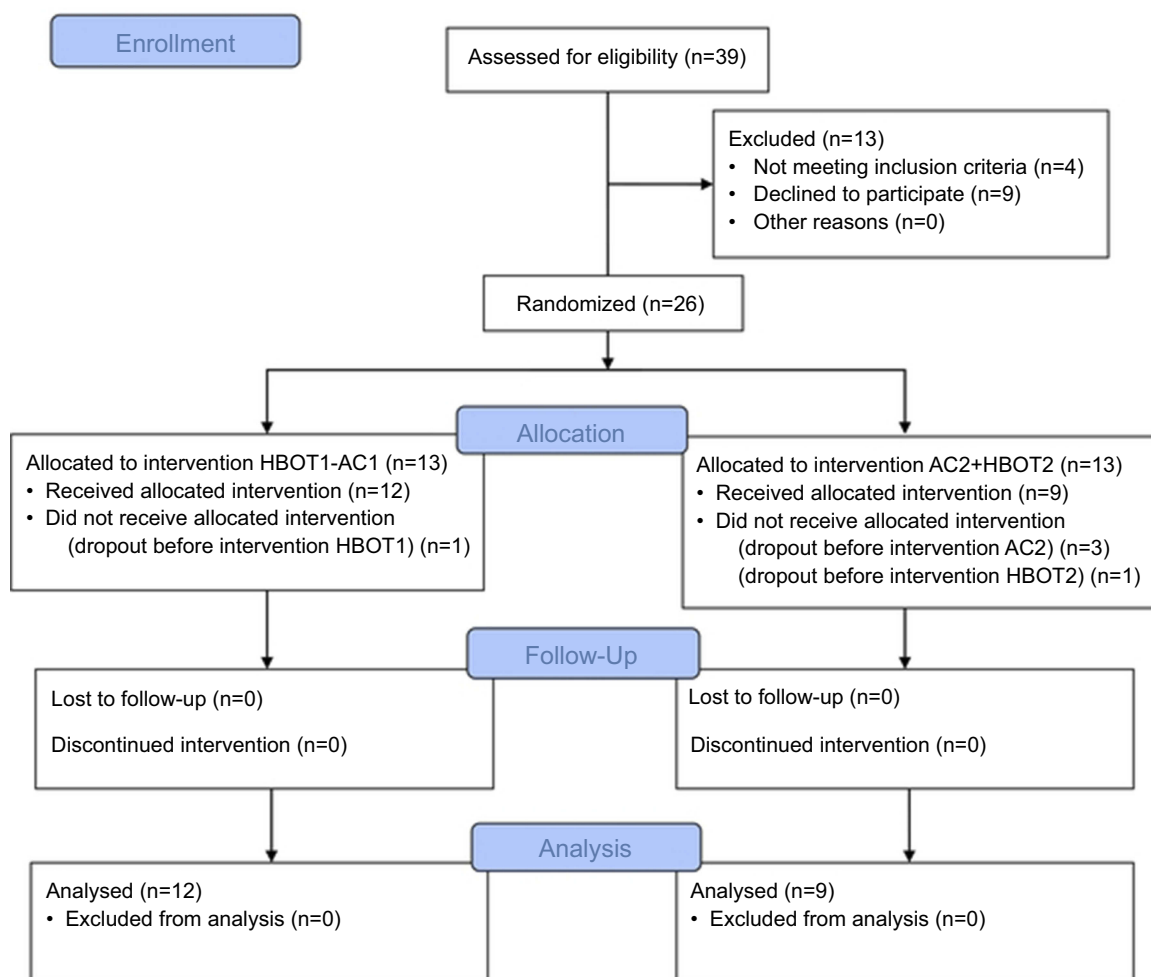
### Study design

A randomized (1:1 block allocation), controlled, crossover, single-blinded design was used. Details are in [Supplemental Document 1](#), [Supplemental Figure 1](#), [Supplemental Table 1](#) and [Supplemental Video 1](#). The subjects were randomized to hyperbaric oxygen treatment (HBOT: 2.4 ATA, 90 min, no air-breaks) or treatment in an open environment during ambient pressure conditions (ambient pressure conditions [AC]: 1 ATA).

### Subjects

Subjects were recruited using [www.forsoeegsperson.dk](http://www.forsoeegsperson.dk). For inclusion and exclusion criteria see [Supplemental Table 2](#). Before inclusion, the subjects received written and oral study information and provided informed written consent. A physical health examination including the completion of a Professional Association of Diving Instructors (PADI) health declaration was performed by a senior medical specialist in diving- and hyperbaric medicine (OH).

All treatment sessions were made at the Hyperbaric Unit, Department of Anesthesia, Head and Orthopedic Center, Rigshospitalet, Copenhagen University Hospitals. The subjects received a compensation of USD 316 (EUR 268) after completion of both sessions.



**Figure 1** CONSORT flow diagram showing the algorithm for enrollment and allocation of subjects.

**Notes:** The intention-to-treat (ITT) number was 26, and the per-protocol (PP) number was 19 subjects.

**Abbreviations:** AC, ambient pressure conditions; HBOT, hyperbaric oxygen therapy.

## Study algorithm

The study included two sessions, identical except for the type of intervention treatment (Figure 2A). Each session started with baseline measurements, in chronological order, skin-erythema (SE) and dermal-thickness (DT), and, assessments of quantitative sensory testing (QST) variables: mechanical thresholds and thermal thresholds. Baseline assessments were followed by a standardized first-degree BI.<sup>14,15</sup> Following the intervention treatment (HBOT/AC) assessments were repeated at timepoints 130, 190 and 250 min (Figure 2A). The two sessions were separated by an interval of  $\geq 28$  days to minimize the risk of carry-over effects from the previous BI.

## Randomization procedure

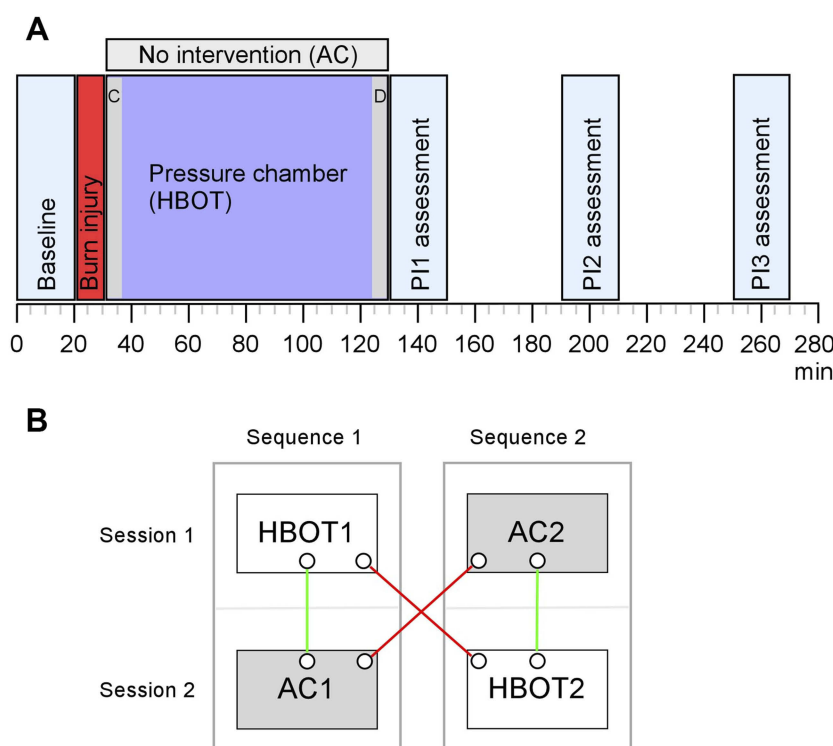
Two randomizations were made using [www.randomizer.org](http://www.randomizer.org). The *first* randomization decided the order of treatment. Subjects were separated into the two sequences; one

sequence starting with the HBOT-session and one starting with the AC-session (Sequence 1: HBOT1-AC1 sequence; Sequence 2: AC2-HBOT2 sequence; Figure 2B), dividing the study into sessions and sequences. The *second* randomization decided upon the left- or right-sided location of the *first* of the two BIs.

Randomization allocations and master randomization lists were kept concealed in opaque envelopes marked with consecutive numbers. Randomization procedures were conducted by a medical staff member, otherwise not associated with this study. The sealed envelopes were opened, when appropriate by the **unblinded** co-examinators (DB, ISN) and allocation instructions were followed.

## Hyperbaric oxygen therapy procedure

The HBOT procedure was performed as previously reported in detail.<sup>8</sup> The procedure consisted of an initial



**Figure 2 (A and B)** Study setup.

**Notes:** (A) Study algorithm. Each session followed the same study algorithm except for type of intervention: baseline assessments (0–20 min); burn injury (20–30 min); HBOT/AC-intervention (30–130 min); and P11–3 including measurements of skin-erythema (SE) and dermal-thickness (DT), and QST assessments, mechanical and thermal thresholds (130–150 min, 190–210 min, 250–270 min). HBOT-intervention included a compression phase (C; 30–35 min); a therapy phase of 100% O<sub>2</sub>; 2.4 ATA (35–125 min); and a decompression phase (125–130 min). AC-intervention included control-therapy with 21% O<sub>2</sub>, 1.0 ATA (30–130 min). Each subject received both treatments during two individual sessions but was randomized to either HBOT1-AC1 or AC2-HBOT2 sequence (Figure 2B). (B) Sequences, Sessions, and within-/between-sequence comparisons. Subject-flows were divided into "sequences" (Sequence 1: HBOT1+AC1; Sequence 2: AC2+HBOT2) and "sessions" (Session 1: HBOT1+AC2; Session 2: AC1+HBOT2). Within-sequence comparisons are comparisons of data within the same sequence (green lines) (HBOT1 vs AC1; AC2 vs HBOT2) whereas the between-sequence comparisons are comparisons of data between sequences (red lines) (HBOT1 vs HBOT2; AC1 vs AC2).

**Abbreviations:** AC, ambient pressure conditions; HBOT, hyperbaric oxygen therapy; PI, post-injury; QST, quantitative sensory testing.

compression period lasting 5 min (Figure 2A), reaching a plateau of 2.4 ATA during which HBOT was administered for 90 min (100% O<sub>2</sub>) using no air-breaks, followed by a short decompression period (Figure 2A).

## Sensitization method

The BIs were induced by the co-examiner using a Peltier-based contact thermode (active area 2.5×5.0 cm<sup>2</sup>, 47°C, 420 s; Thermotest, MSA, Somedic AB, Hörby, Sweden). The thermode was positioned at a prespecified site on the calf, using the homologous anatomical site on the contralateral side during the second session. An elastic compression bandage kept the thermode in position maintaining a constant application pressure during all assessments. The subject rated the intensity of pain, using a horizontally held, visual analog scale (VAS; 0= no pain, 10= worst imaginable pain) at baseline, and, at timepoints 0, and 15, 30, 45, 60, 120, 180, 240, 300, 419 and 425 s after the thermode had reached 47°C.

## Measurements and assessments

### Skin-erythema and dermal-thickness

Measurements of SE and DT were made inside and outside the testing area (10 cm distal from the center) using a combined non-invasive, skin-reflectance spectrophotometer and a high-resolution ultrasound scanner (Derma-Lab Combo, Cortex Technology ApS, Hadsund, Denmark).<sup>16,17</sup> The spectrophotometer was calibrated prior to all assessments. The SE was measured as the erythema index (arbitrary units), and the DT was measured in the testing area as previously described.<sup>15</sup> Measurements were each made in triplicates using the mean value in further analysis.

### Mechanical thresholds

Assessments of pin-prick pain threshold (PPT) were made in the testing area using "weighted-pins" (8, 16, 32, 64, 128, 256 and 512 mN; MRC Systems, Heidelberg, Germany) according to a modified Dixon's "up-and-down method",<sup>18–20</sup> as



previously reported.<sup>8</sup> The PPT was determined four times, and the median value was used in further analysis.

### Secondary hyperalgesia areas

Assessment of the SHA was by a 512 mN “weighted-pin” stimulator.<sup>19</sup> The area surrounding the BI was stimulated clockwise along eight symmetrical radials converging towards the center of the BI, as previously reported.<sup>8</sup> The stimulation began in normal skin with an application rate of 0.5 Hz and a distance of 1–2 cm between each pin-prick, projecting in towards the center. The subject indicated when a definite change in perception, from a non-noxious to an uncomfortable or stinging sensation occurred. The octagonal corners of secondary hyperalgesia were marked on the skin. The markings were then transferred by manual copying onto a transparent overhead sheet and digitally transferred to a vector-based computer program (Canvas 8.0, ACD Systems International, Victoria, Canada) for area calculations. The SHA were calculated by subtracting the testing area from the total area.

### Thermal Thresholds

Warmth detection threshold (WDT), cool detection threshold (CDT) and heat pain threshold (HPT) were assessed using the contact thermode system and determined according to the method-of-limits,<sup>21</sup> as previously reported.<sup>8</sup> Threshold assessments were made in triplicate using the mean value in further analysis.

## Statistics

### Statistical analyses

Data were manually checked for errors by one of the investigators (AW) and by the GCP-unit. Statistical analyses were made using the MedCalc Statistical Software (Version 15.11.4, MedCalc Software bvba, Ostend, Belgium). During the statistical analyses, data were first partially unblinded dividing the subjects into two groups, A and B. Data were unblinded only after completion of the analysis. Normality of the data was tested using the Kolmogorov-Smirnov test and visual inspection of residual plots. When relevant, data were corrected with baseline values obtained at the beginning of each session to avoid potential carryover effects between the two sessions. To avoid mass significance in multiple comparisons, a summated AUC-measure (area-under-the-curve  $\text{min}^{-1}$ ) was used where appropriate. For normally distributed data, significance was determined using the paired or unpaired t-tests, and for non-normally distributed data the

Wilcoxon or Mann-Whitney tests, respectively, were used as appropriately. Comparisons across sessions and sequences were made to explore potential preconditioning effects (Figure 2B). In analysis of the consistency of baseline assessments, intraclass correlation coefficients (ICC [2,1; two-way random single measures, consistency]) were used, categorized as previously reported.<sup>22</sup> Depending on normality, data are presented as mean or median (95% CI). The level of statistical significance was *a priori* set at  $<0.05$ . The Bonferroni correction was used for multiple comparisons for the primary outcome, the statistical significance therefore was set at 0.025.

### Sample size estimates

Based on the primary outcome, SHA, and the results from our original study,<sup>8</sup> an *a priori* sample size estimation was made using a minimal relevant difference of 19  $\text{cm}^2$  and a standard deviation of 28  $\text{cm}^2$ . The significance level was set at 0.05 ( $\alpha$ ) and the power at 0.8 ( $\beta=0.2$ ). A randomized, single-blinded, crossover design was used. The calculations yielded an estimated sample size of 19 per-protocol subjects, but to compensate for dropouts the number of intention-to-treat subjects was 26.

## Exploratory analyses of combined data

Exploratory analyses were made by combined data from the present study with data from our original study.<sup>8</sup> Statistical analyses were made using the same methods as described in the previous paragraph.

## Results

### Subjects

#### Subject flow

The first study session was on February 16, 2015, and the last study session was on May 13, 2015. The study was completed when the *a priori* estimated sample size had been reached. Twenty-six subjects were included in the study following the initial visit, however, five subjects did not complete the study (Figure 1; CONSORT 2010 Flow Diagram). Two subjects (#3, #6) never showed up after the initial visit. One subject (#26) decided, due to time restraints, not to take part in the study. One subject (#13) had to withdraw before the intervention, due to medical reasons not associated with the study. One subject (#1) never showed up at the second study session (HBOT2-session). Data from these five subjects were not included in the statistical analyses. Thus, 21 subjects completed the trial. No adverse effects or complications were seen during the study.

## Missing data

Data sets from these 21 subjects were incomplete for subjects #10, #16 and #25. Subjects #10 and #16 were unable to consistently perceive secondary hyperalgesia, and their SHA-assessments were excluded from the analysis, leaving 19 data sets available for the primary outcome analysis. Subject #25 presented an incomplete data set regarding SE-measurements, leaving 20 data sets for analysis of SE-data ( $n=20$ ). The missing SE-data were attributed to human error, constituting only 4/3,864 (0.1%) of the total number of data entries.

## Anthropometrics

Anthropometrics are shown in Table 1.<sup>38</sup>

## Burn injury

The median time interval between the two BIs (sessions) was 35 days (34–39 days). The mean pain intensity during the BIs, calculated as AUC, was 3.3 (2.4–4.1) VAS-units during the AC-sessions, and 3.0 (2.3–3.6) VAS-units during the HBOT-sessions ( $P=0.59$ ).

## Primary outcome (secondary hyperalgesia areas)

### Baseline data

Data consistency during Session 1 vs Session 2 (Figure 2B) was examined by ICC's (0.85 [0.64–0.94]) and by comparisons of baseline assessments using simple paired sample tests demonstrating a highly significant difference in baseline assessments between the sessions (Wilcoxon:  $P=0.0017$ ). The median values for SHA were significantly larger in Session 1 (26.6 [12.5–37.6]  $\text{cm}^2$ ) compared to Session 2 (15.9 [12.5–23.4]  $\text{cm}^2$ ).

**Table 1** Anthropometric data

	ITT; $n=21$	PP; $n=19$
Height (cm)	183.2 (179.5–186.9)	183.5 (179.5–187.6)
Weight (kg)	79.8 (74.6–84.9)	79.2 (73.6–84.8)
Age (yr) <sup>a</sup>	26.1 (24.9–29.5)	26.1 (24.7–28.8)
BSA ( $\text{m}^2$ )	2.01 (1.93–2.09)	2.01 (1.92–2.09)
BMI ( $\text{kg}/\text{m}^2$ )	23.7 (22.5–24.9)	23.5 (22.2–24.7)

**Notes:** Data are normally distributed (mean [95% CI]), except for age <sup>a</sup>(median [95% CI]).

**Abbreviations:** ITT, intention-to-treat; PP, per-protocol; BMI: body mass index; BSA: body surface area.

## Post-injury vs baseline assessments

Data consistency of BI-induced changes in sensory assessments were examined by comparisons between baseline and post-injury 1–3 (PI1–3) AUC-values. Significantly increased BI-induced SHA-values were seen in both sessions (HBOT-session,  $P=0.0002$ ; AC-session,  $P=0.0001$ ).

HBOT vs AC-sessions: SHA-values of the HBOT- and AC-sessions, respectively, were calculated as AUC *corrected* for the respective baseline values and given as the mean. HBOT-sessions showed a SHA of 18.8 (10.5–27.0)  $\text{cm}^2$ , whereas the AC-sessions had an area of 32.0 (20.1–43.9)  $\text{cm}^2$  ( $P=0.021$ ; Figure 3A), indicating a significant mitigating effect of HBOT-sessions compared to AC-sessions.

## Within-sequence and between-sequence effects

Within-sequence effects were examined, in Sequence 1 comparing session HBOT1 with session AC1, and, in Sequence 2 comparing session AC2 with session HBOT2 (Figure 2B). The comparison in Sequence 1 (HBOT1 vs AC1; Figure 4A) did not yield any statistical difference ( $P=0.55$ ) while in Sequence 2 (AC2 vs HBOT2), a significantly larger area in the AC2-session was demonstrated, corresponding to a mean difference of 25.0 (5.4–44.7)  $\text{cm}^2$ , compared to the HBOT2-session ( $P=0.019$ ).

Between-sequence effects were examined by comparing session HBOT1 with session HBOT2, and, by comparing session AC1 with session AC2 (Figure 2B). While the comparison of HBOT-sessions did not yield any statistical significance ( $P=0.73$ ), comparison of the AC-sessions demonstrated a statistically significant larger area in the AC2-session, corresponding to a mean difference of 25.5 (4.9–46.1)  $\text{cm}^2$ , compared to the AC1-session ( $P=0.018$ ).

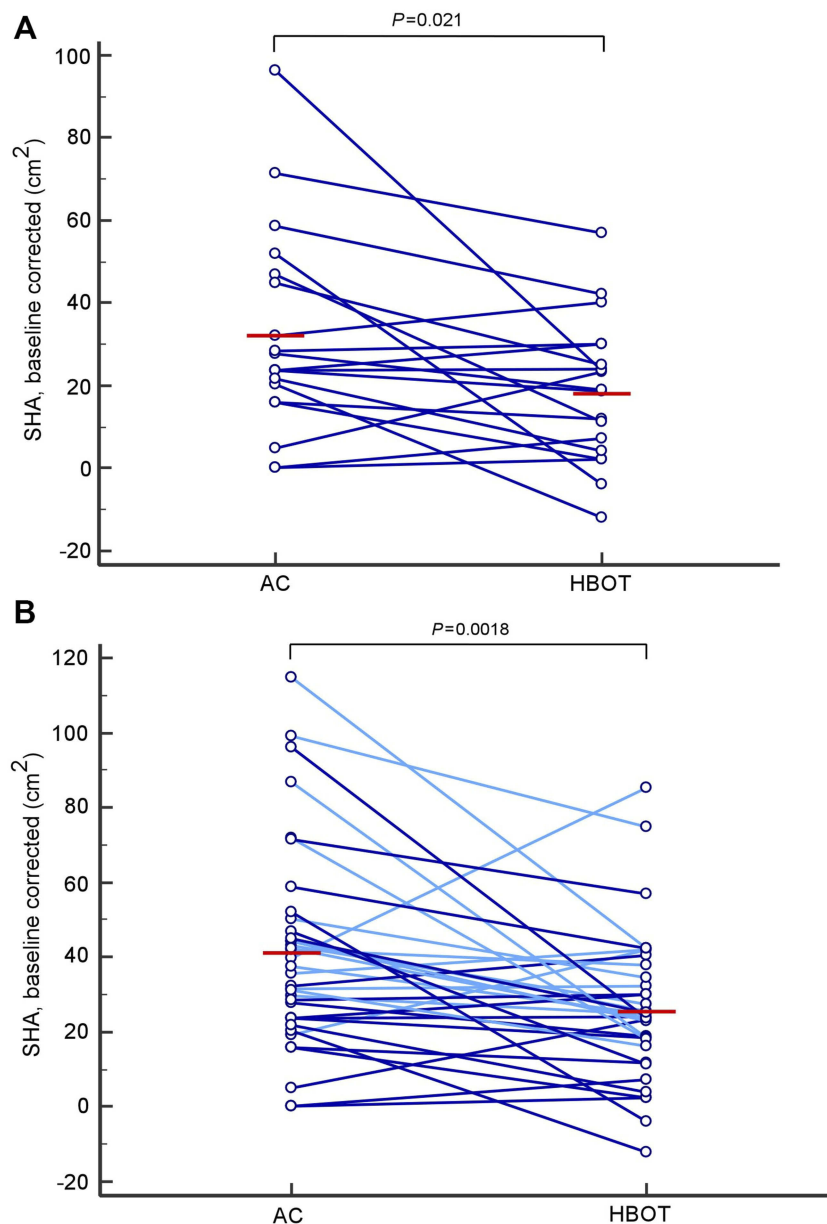
## Summary

The analyses demonstrated a mitigating effect on SHA of HBOT-sessions *vis-à-vis* AC-sessions. Furthermore, they demonstrated a significant preconditioning effect in Sequence 1 when the HBOT-session was administered prior to the AC-session, compared to Sequence 2, when the AC-session preceded the HBOT-session (Figure 2B).

## Secondary outcomes (SE, DT, mechanical and thermal thresholds)

### Baseline data

Data consistency for secondary outcomes are available in Supplemental Document 2.



**Figure 3 (A and B)** Changes in secondary hyperalgesia areas.

**Notes:** (A) Present study ( $n=19$ ). Dot-line diagram illustrating the individual subject's changes in secondary hyperalgesia areas (SHA) during ambient pressure conditions sessions (AC) and hyperbaric oxygen therapy sessions (HBOT). The mean values of AC- and HBOT-sessions were  $32.0 \text{ cm}^2$  ( $20.1\text{--}43.9 \text{ cm}^2$ ) and  $18.8$  ( $10.5\text{--}27.0 \text{ cm}^2$ ; paired  $t$ -test:  $P=0.021$ ), respectively, indicated by red lines. SHA-values are baseline corrected, calculated as AUC and presented as  $\text{cm}^2$ . Negative values are results of corrections with baseline values in two subjects perceiving a baseline, pre-burn SHA (cf. "Protocol violations"). (B) Combined data ( $n=36$ ). Dot-line diagram illustrating the combined data. The mean values of AC- and HBOT-sessions were  $40.9 \text{ cm}^2$  ( $31.8\text{--}50.0 \text{ cm}^2$ ) and  $26.4$  ( $19.7\text{--}33.0 \text{ cm}^2$ ; paired  $t$ -test:  $P=0.0018$ ), respectively, indicated by a red line.

**Abbreviations:** AC, ambient pressure conditions; HBOT, hyperbaric oxygen therapy; SHA, Secondary hyperalgesia areas.

### Post-injury vs baseline assessments

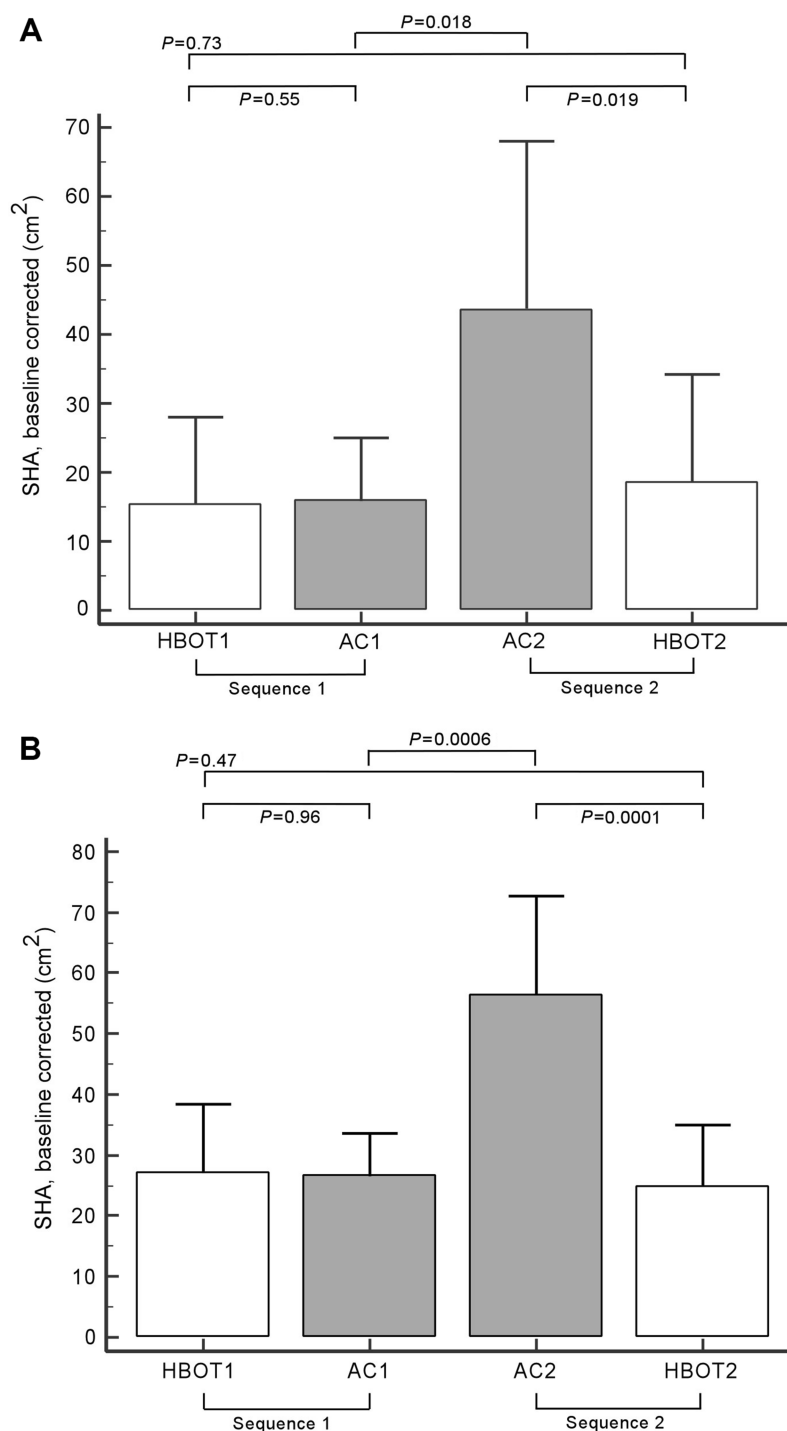
Comparisons between baseline values and post-injury values are available in [Supplemental Document 2](#).

### HBOT vs AC-sessions

No statistically significant differences between sessions, calculated as AUC *corrected* for the respective baseline

values, were found regarding the mean values in SE, DT, PPT or the median values in CDT. A significantly lower mean WDT was found in the HBOT-session compared to the AC-session ( $P=0.011$ ). A significantly lower mean value was also found regarding HPT in the HBOT-session, compared to the AC-session ( $P=0.045$ ). For detailed secondary outcome analyses see [Supplemental Document 2](#).





**Figure 4 (A and B)** Protective, preconditioning effect of hyperbaric oxygen therapy on secondary hyperalgesia areas.

**Notes:** (A) Present study (n=19). Box-plots of secondary hyperalgesia areas (SHA; mean [95% CI]) during Sequence 1 (HBOT1-AC1) and Sequence 2 (AC2-HBOT2). The SHA are baseline corrected, calculated as AUC and presented as cm<sup>2</sup>. A significant difference between the HBOT- and AC-sessions was seen in Sequence 2 ( $P=0.019$ ), indicating a preconditioning effect of HBOT. No difference was seen between the HBOT- and AC-sessions in Sequence 1 ( $P=0.56$ ). (B) Combined data (n=36). A significant difference between the HBOT- and AC-sessions was seen in Sequence 2 ( $P=0.0001$ ), indicating a preconditioning effect of HBOT. No difference was seen between the HBOT- and AC-sessions in Sequence 1 ( $P=0.55$ ). Data were compared using the paired t-test.

**Abbreviations:** AC, ambient pressure condition sessions; AUC, area-under-the-curve; HBOT, hyperbaric oxygen therapy session; SHA, Secondary hyperalgesia areas.

## Combined primary outcome data from original and present study

### Subjects

Nineteen subjects were included from the present study and 17 subjects from the original study,<sup>8</sup> making a total of 36 subjects available for the combined data analysis.

### Baseline data

The original study showed no baseline SHA and, therefore, the combined baseline data are identical to results of the present study.

### Post-injury vs baseline assessments

Significantly increased BI-induced SHA-values were seen in both sessions (HBOT-session and AC-session,  $P=0.0001$ ).

### HBOT vs AC-sessions

Significantly smaller mean SHA were found in the HBOT-group (26.4 [19.7–33.0]; **Figure 3B**) compared to the AC-group (40.9 [31.8–50.0];  $P=0.0018$ ), corroborating a mitigating effect of HBOT.

### Within-sequence and between-sequence effects

Within-sequence effects showed no statistical difference in Sequence 1 (HBOT1 vs AC1; **Figure 4B**) ( $P=0.96$ ) while significantly larger SHA were found in the AC2-session compared to the HBOT2-session, corresponding to a mean difference of 30.5 cm<sup>2</sup> (17.9–43.2 cm<sup>2</sup>;  $P=0.0001$ ).

Between-sequence effects comparing the HBOT-sessions (HBOT1 vs HBOT2; **Figure 4B**) did not yield any statistical difference ( $P=0.47$ ). However, a significant difference was found when comparing the AC-sessions (AC1 vs AC2) showing a statistically significantly larger area in the AC2-session, corresponding to a mean difference of 29.7 cm<sup>2</sup> (14.9–44.4 cm<sup>2</sup>;  $P=0.0006$ ), compared to the AC1-session, corroborating the preconditioning effect of the HBOT1-session.

### Summary

The analyses of the combined data demonstrated *first*, a highly significant decrease in SHA related to HBOT compared to AC ( $P=0.0018$ ). *Second*, a highly significant preconditioning effect on the development of SHA related to HBOT ( $P=0.0001$ ). *Third*, the combined study results corroborated the data consistency between the studies.

## Protocol violations

During data collection, the examiner noticed a number of baseline pre-burn SHA. However, according to our experience, this is rarely observed in healthy skin, and therefore a short questionnaire was made to evaluate if the subjects were, in fact, able to detect SHA. Most subjects were able to discriminate secondary hyperalgesia from normal sensation. However, two subjects (#10, #16) were not able to discriminate or could not recall a perceptual difference between pre- and post-burn SHA and were therefore excluded in the data analysis of SHA (cf. *Missing data*). The implementation of the questionnaire had not been described in the protocol and is, therefore, a minor violation of protocol.

## Discussion

In the human experimental BI-model, we demonstrated that HBOT mitigates inflammation-induced secondary hyperalgesia. Remarkably, a single session of HBOT also has a preconditioning, long-lasting, pre-emptive anti-hyperalgesic effect on a contralateral repeat injury. Since no signs of mitigation of the primary hyperalgesia in the injured areas were found, our data clearly demonstrate that HBOT has acute and long-lasting neuroplasticity effects on central sensitization.<sup>23,24</sup>

## Mitigation of secondary hyperalgesia

Secondary hyperalgesia is a centrally induced phenomenon<sup>12,13,23</sup> where the conditioning input from nociceptors by heterosynaptic potentiation amplifies the response from segmentally connected afferent non-nociceptive and nociceptive nerve fibers. Although the clinical role of central sensitization has been debated,<sup>23,25</sup> it may contribute to the pain trajectories of osteoarthritis, temporomandibular joint disorders, neuropathic pain, visceral pain hypersensitivity disorders and persistent post-surgical pain.<sup>12</sup> Neuroplasticity represents a physiological adaption in the nervous system responding to perturbations in the external environment.<sup>26,27</sup> Neuroplasticity leads to functional and structural alterations in the nervous system and is intimately linked to memory processing, storage and consolidation. In the nociceptive system, modulation of ascending and descending control pathways are by neuroplasticity mechanisms.<sup>28</sup> In humans central sensitization of pain, eg, secondary hyperalgesia, temporal summation, and long term potentiation,<sup>12,29</sup> is often coined a

maladaptive neuroplasticity response, mainly affecting the descending inhibition system.<sup>27,28</sup> The available evidence from this study, therefore, substantiate the presence of a potential neuroplasticity mechanism in the phenotypic changes: the mitigation of secondary hyperalgesia. However, it would be interesting to obtain further proof of neuronal plasticity with MRI using the BOLD-technique.<sup>30</sup>

Interestingly, the time frame of neuroplasticity changes in pain phenotype is neatly demonstrated in the present study. The acute mitigating effect on injury induced SHA is seen hours after the injury while the preemptive, protective effect on development of SHA is demonstrated four weeks following preconditioning with a single session HBOT. Recent clinical studies indicate that HBOT attenuates chronic pain in fibromyalgia by inducing neuroplasticity following a series of 40 treatment sessions, thereby rectifying abnormal brain activity in pain related areas.<sup>3</sup> One animal study found a two-phase antinociceptive effect, with the second phase lasting up to three weeks after four HBOT-sessions by a mechanism mediated through the NO dependent release of endogenous opioids.<sup>31</sup> As pain disorders are not congenital but evolve over time due to multifactorial causes, the concept of neuroplasticity is inherent in the development of pain disorders. Since HBOT has been shown to induce neuroplasticity in the chronically injured brain even months to years after the acute insult,<sup>3,32</sup> understanding the specific mitigating mechanisms of HBOT on the pathophysiological perturbations following tissue injury may shed some light on future management strategies as well as indicating rational and targeted use of HBOT<sup>33</sup> in various pain disorders.

## Effects of HBOT on the primary burn injury area

A reduction in WDT and HPT in the HBOT-sessions compared to the AC-sessions was demonstrated, but no other significant differences in QST-indices in the primary BI-area were found. Multiple comparisons were made in the secondary analysis without using Bonferroni correction, leaving a considerable risk of type 1 error. Therefore, our results hardly support any effect of HBOT on the primary BI-area.

## Combined data from original and present study

### Methodological differences

We decided to combine the data of the present study with data from our original study<sup>8</sup> in order to cautiously examine

the statistical consistency of the studies. The two studies are methodologically almost identical, but few important differences do exist. *First*, the roles of examiner and co-examiner, and, the blinding procedure, are novel in the present study. *Second*, the delineating-method of SHA using pin-prick instruments vs polyamide monofilaments in the original study differed. However, this unlikely influences the results, since we used paired data analysis with each subject being his control and furthermore, it has been demonstrated that the results obtained by the two delineating-methods are reproducible and inter-correlated.<sup>19</sup> We, therefore, consider combining the results to be appropriate and methodologically interesting.

### Mitigation of secondary hyperalgesia and preconditioning

When combining the data, the results had the same general tendency as in the individual studies, but with a markedly increased statistical significance. Although the interpretation of *post-hoc* analyses should be made with diligence the effect sizes of the present study and the combined studies, were very large, 2.5 and 3.5, respectively. The statistical significance increased with *P*-values from 0.021 (n=19) to 0.0018 (n=36), making a type I error very unlikely.

### Data consistency

In the present study and the combined studies, we found a statistically significant difference in SHA baseline values between Session 1 and Session 2, probably indicating a habituation phenomenon. However, this does not affect our results, since corrections with baseline values have been made. The ICC's showed excellent data consistency.

## Limitations of the study

### Blinding conditions

During this single-blinded, block-randomized, controlled, crossover study, the examiner was blinded to the subject's allocation, but the design did not prevent the development of subject bias. While it is highly unlikely that the subject could consciously replicate the preconditioning effects with the sparse information received in combination with the randomization- and group allocation procedures performed (Figure 2B), we cannot exclude subject bias. Double-blind designs have been reported to be feasible in a hyperbaric environment.<sup>1,34,35</sup> However, to do this, a single-seat hyperbaric chamber would be needed or, alternatively, a study design with seven participants receiving treatment simultaneously requiring a huge number of

examiners and co-examiners to do the assessments. Unfortunately, neither of these setup designs were possible during the present study.

### Multiple comparisons

Multiple comparisons were made in the statistical sub-analyses of the primary outcome increasing the likelihood of introducing a type 1 error. In spite of the conservative Bonferroni correction, the results continued to be statistically significant in the present study and the combined analyses, indicating that a type 1 error would be highly unlikely.

### Advantages of the study

Growing concerns about the reproducibility in scientific research has been expressed during the last decade.<sup>36,37</sup> In the previous study the small number of subjects and the single-session procedure seemed potential confounding factors. Our principal objective of the present study was to replicate and validate our previous study results on the effect of HBOT on human burn pathophysiology.

### Conclusion

The present study demonstrates that hyperbaric oxygen therapy has an immediate mitigating effect, as well as a long-lasting preconditioning effect, on secondary hyperalgesia induced by a repeat burn injury. These are the first studies in humans to demonstrate ameliorating effects of hyperbaric oxygen therapy on central sensitization, and the results corroborate experimental findings from animal studies. Hyperbaric oxygen therapy seems an interesting venue for future preventive measures in severe pain conditions characterized by central sensitization, eg, persistent postsurgical pain and phantom limb pain.

### Abbreviation list

AC, ambient conditions; AUC, area-under-the-curve per minute; BI, burn injury; CDT, cool detection threshold; DT, dermal-thickness; HBOT, hyperbaric oxygen therapy; HPT, heat pain threshold; ICC, intraclass correlation coefficients; PADI, Professional Association of Diving Instructors; PPT, pin-prick pain threshold; SE, skin-erythema; SHA, secondary hyperalgesia areas; WDT, warmth detection threshold.

### Data availability

The complete dataset is available in [Supplemental File 1](#).

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### Author contributions

AW, MW and PRN conceived the study. AW, DB, MW, OH and PRN designed the study. AW, DB and ISN conducted the study. MW, OH and PRN supervised the study. AW and MW analyzed and interpreted data, performed statistical analyses and wrote the manuscript. All authors critically revised the manuscript, gave final approval for publication and agree to be accountable for all aspects of the work.

### Disclosure

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